

Why is it critical to achieve a deep molecular response in chronic myeloid leukemia?

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ABSTRACT

The primary goal of tyrosine kinase inhibitor (TKI) therapy for patients with chronic myeloid leukemia is survival, which is achieved by the vast majority of patients. The initial response to therapy provides a sensitive measure of future clinical outcome. Measurement of *BCR-ABL1* transcript levels using real-time quantitative polymerase chain reaction standardized to the international reporting scale is now the principal recommended monitoring strategy. The method is used to assess early milestone responses and provides a guide for therapeutic intervention. When patients successfully traverse the critical first 12 months of TKI therapy, most will head towards another milestone response, deep molecular response (DMR, *BCR-ABL1* $\leq 0.01\%$). DMR is essential for patients aiming to achieve treatment-free remission and a prerequisite for a trial of TKI discontinuation. The success of discontinuation trials has led to new treatment strategies in order for more patients to reach this milestone response. DMR has been incorporated into end-points of clinical trials and is considered by some expert groups as the optimal treatment response. But is DMR a stable response and does it provide the ultimate protection against TKI resistance and death? Do we need to increase the sensitivity of detection of *BCR-ABL1* to better identify the patients who would likely remain in treatment-free remission after TKI discontinuation? Is it necessary to switch current TKI therapy to a more potent inhibitor if the goal is to achieve DMR? These are issues that I will explore in this review.

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Introduction

It has been 20 years since the first patients with newly diagnosed chronic myeloid leukemia (CML) were treated with imatinib in the International Randomized Study of Interferon and STI571 (IRIS) trial.¹ The primary endpoint of that pivotal trial was the rate of progression, which included death, blast crisis or accelerated phase, loss of complete hematologic response, loss of major cytogenetic response or an increasing white cell count. Back then, in 2000, response was measured at the hematologic and cytogenetic levels. How things have changed for patients diagnosed with CML! Treatment response is now principally monitored at the molecular level by quantitative measurement of *BCR-ABL1* transcripts.

The prognostic value of molecular monitoring in patients treated with a tyrosine kinase inhibitor (TKI) was first demonstrated in the IRIS trial.² The analysis was exploratory and in general performed only after the achievement of a complete cytogenetic response. The majority of imatinib-treated patients did indeed achieve a complete cytogenetic response and the term 'major molecular response' (MMR) was coined. This response was subsequently described as a 'safe haven' in which the risk of loss of response is low.³ The rate of MMR is now a primary or secondary outcome measure in CML clinical trials^{4,5} and is measured on an international reporting scale (IS) ($\leq 0.1\%$ *BCR-ABL1* IS).⁶ A deep molecular response (DMR, *BCR-ABL1* $\leq 0.01\%$ IS) is also now an important milestone since it is a prerequisite for a trial of drug discontinuation with the aim of achieving treatment-free remis-

sion (TFR). The safety of TKI discontinuation for patients who achieve and maintain a DMR has been demonstrated in multiple clinical trials⁷⁻¹⁶ and international expert groups have incorporated TKI discontinuation into their recommendations and guidelines.¹⁷⁻²⁰

The majority of CML patients will require lifelong TKI treatment and long-term molecular monitoring is recommended for all patients with CML.¹⁸⁻²⁰ The European LeukemiaNet (ELN) mandates measurement of *BCR-ABL1* transcripts according to the IS at least 3 monthly, even after MMR is confirmed because close monitoring is required to assess eligibility for TKI discontinuation.¹⁹ Most patients will eventually achieve a DMR.^{21,22} Whether or not DMR predicts survival has been debated and it has not been confirmed that it does.²²⁻²⁴ Achieving a sustained DMR is a prerequisite for TKI discontinuation, but is it also a biomarker for better clinical outcomes?^{23,25}

Is deep molecular response the optimal molecular response for patients with chronic myeloid leukemia?

In 2013, the ELN incorporated molecular monitoring using standardized real-time quantitative polymerase chain reaction (qRT-PCR) analysis into their recommendations for the management of CML.²⁶ An optimal response was *BCR-ABL1* $\leq 10\%$, $\leq 1\%$ and $\leq 0.1\%$ at 3, 6 and 12 months of TKI therapy. Treatment failure was defined as *BCR-ABL1* $> 10\%$ at 6 months and $> 1\%$ at 12 months. These recommendations were based on strong evidence collected over many years²⁷⁻³⁵ and subsequent studies consolidated the recommendations.³⁶⁻⁴¹ The importance of a one-log reduction of *BCR-ABL1* by 3 months and two-log reduction by 6 months for progression-free survival was reported as early as 2003.^{28,29} The equivalent *BCR-ABL1* transcript values on the IS are 10% and 1%, respectively. An update of molecular data generated in the IRIS trial was published in 2010, and used to examine the prognostic significance of early molecular response.³⁰ Landmark analyses of *BCR-ABL1* values at 6, 12 and 18 months of imatinib therapy established that event-free survival was inferior for patients with $> 10\%$ at 6 months and $> 0.1\%$ at 12 and 18 months. Progression to accelerated phase or blast crisis and overall survival were inferior for patients with *BCR-ABL1* $> 10\%$ at 6 months, and $> 1\%$ at 12 and 18 months.³⁰ In 2012, Hanfstein *et al.*³¹ and Marin *et al.*³² confirmed the strong association between *BCR-ABL1* values at 3, 6 and 12 months and outcome. Marin *et al.* reported that the *BCR-ABL1* value at 3 months was the only requirement for predicting outcome for patients treated with a TKI.³² Furthermore, a *BCR-ABL1* value of $\leq 0.61\%$ at 3 months was highly predictive of subsequent undetectable *BCR-ABL1*. The cumulative incidence of undetectable *BCR-ABL1* at 8 years for patients with *BCR-ABL1* $\leq 0.61\%$ was 84.7% whereas it was 1.5% for those with $> 0.61\%$ ($P < 0.001$).³² This study highlighted the importance of rapid leukemic clearance for a subsequent DMR.

The 2013 ELN recommendations for the management of CML²⁶ were the first time that molecular response was incorporated into therapeutic decisions by an expert group, although quite wisely, caution was advised regarding the interpretation of the molecular values. An additional molecular test was recommended to confirm treatment failure. Numerous publications had confirmed the predictive value of molecular monitoring,²⁷⁻³⁵ but most of the studies had been performed in academic centers with

long-term experience in molecular monitoring. The ELN recognized that the standard of testing in these studies may not have represented the typical standard at that time.

Widespread incorporation of molecular monitoring for clinical decisions was made possible by the introduction of the standardized IS for *BCR-ABL1*. This was coupled with harmonization of testing processes, standardization of the nomenclature for reporting molecular response and the development of reference material.⁴²⁻⁵⁰ The term complete molecular response was replaced by MR4 (*BCR-ABL1* $\leq 0.01\%$ IS) and MR4.5 (*BCR-ABL1* $\leq 0.0032\%$ IS).⁴⁷ These terms apply to both detectable and undetectable *BCR-ABL1* and incorporate the sensitivity achieved for individual samples. However, method standardization has been challenging and regular molecular monitoring on the IS is by no means available to all patients because economic circumstances may hinder its widespread use.^{51,52} Nevertheless, molecular monitoring is the principal recommended monitoring strategy.¹⁸⁻²⁰ Furthermore, in countries with the most advanced standardized monitoring programs, multicenter, high-quality DMR assessment is achievable. This was demonstrated in a recent study conducted by the European Treatment and Outcome Study (EUTOS) group in which DMR was measured reliably by local laboratories in Europe, not just the key reference laboratories of individual countries.⁵³

There are differences of opinion between experts regarding the early molecular response milestone values. Table 1 compares these values between the recent updated ELN recommendations¹⁹ and the National Comprehensive Cancer Network (NCCN) clinical practice guidelines.²⁰ The NCCN guidelines have less stringent *BCR-ABL1* cut-off values at 6 months ($\leq 10\%$) and 12 months ($\leq 1\%$) for TKI-sensitive disease (no change of therapy required). The ELN cut-off values for an optimal response are $\leq 1\%$ at 6 months and $\leq 0.1\%$ at 12 months (no change of therapy required). Furthermore, the ELN now considers a *BCR-ABL1* value of $\leq 0.01\%$ at any time as the optimal response for patients aiming for TFR. The ELN has a buffer response criterion of 'warning' between each milestone *BCR-ABL1* cut-off value. A recommendation in cases of 'warning' is additional molecular monitoring if the kinetics of response is not clear. The trend of *BCR-ABL1* decline over time can aid clinical decisions.^{54,55} The NCCN also suggests assessing the trend of decline for patients with *BCR-ABL1* only slightly $> 10\%$ at 3 months before making drastic decisions regarding the treatment strategy. The definition of TKI-resistant disease after 12 months of TKI therapy is less stringent in the NCCN guidelines than in the ELN recommendations: *BCR-ABL1* cut-off $> 10\%$ for the NCCN and $> 1\%$ for the ELN (ELN 'Failure' category).

The most recent NCCN guidelines differ from previous versions in which a *BCR-ABL1* value of $\leq 0.1\%$ at 12 months indicated TKI-sensitive disease.⁵⁶ The value is now $\leq 1\%$ at 12 months and TKI-resistant disease is defined as $> 1\%$ at ≥ 15 months.²⁰ However, the NCCN still recognizes the value of MMR at 12 months and a statement is included in the 2020 guidelines: "*BCR-ABL1* 0.1% at 12 months is associated with a very low probability of subsequent disease progression and a high likelihood of achieving a subsequent MR4.0, which may facilitate discontinuation of TKI therapy."²⁰

A recent analysis of the German CML-Study IV con-

firmed the optimal response time to achieve 1% *BCR-ABL1* at about 12 to 15 months for progression-free survival, with progression being development of accelerated phase, blast crisis or death.⁵⁷ The study also investigated when it is necessary to regard lack of MMR as treatment failure, indicating that a switch of therapy is warranted. The landmark time point of 2.5 years to achieve MMR showed the largest difference between those with or without MMR with regard to progression-free survival.⁵⁷ A specific time to achieve DMR for progression-free survival was not detected. The updated ELN recommendations now state a change of treatment may be considered if MMR is not reached by 36-48 months.¹⁹

How stable is deep molecular response?

Multiple issues may contribute to loss of a DMR, including dose reduction, and cessation or non-adherence to therapy. A rise in *BCR-ABL1* levels can be an exquisite indicator of non-adherence.⁵⁸ A very rapid rise can indicate complete lack of kinase inhibition due to abrupt TKI cessation.⁵⁸ Loss of a DMR is rarely associated with drug resistance. A recent single-center review of 450 patients demonstrated that sustained MR4 for at least 12 months represented a secure response threshold.⁵⁹ This finding only applied to compliant patients with no history of previous TKI resistance who received standard-dose TKI. No such patient lost a MMR, whereas loss of MMR occurred in 25% of patients who had not achieved a MR4. Importantly, failure to sustain a MR4 was the only significant variable for loss of MMR in multivariate analysis.⁵⁹ We also found that sustained undetectable *BCR-ABL1* (MR4.5) was associated with sustained MMR.²¹ Conversely, MMR was lost in six of 22 (27%) patients with sustained detectable *BCR-ABL1* and was associated with the acquisition of imatinib-resistant *BCR-ABL1* kinase domain mutations in three of six patients. None of these three patients had achieved a DMR. Similarly, a recent study found *BCR-ABL1* mutations in 26% of patients who lost a MMR, although it is not known whether any of these patients achieved a DMR.⁶⁰

The molecular response levels use defined cut-off values and the inherent variability of the quantitative PCR assay means there will be fluctuations above or below the cut-off values.^{61,62} These fluctuations will be greater at low levels of *BCR-ABL1*. However, in some cases, fluctuations are an indication of subsequent relapse. A study of 208 patients treated with imatinib as their first-line therapy investigated the outcome of patients with fluctuating

BCR-ABL1 values according to the level of response achieved.⁶³ A stable molecular response was defined as persistence of the same molecular response (MMR, MR4 or MR4.5) at three consecutive assessments. A fluctuation was the achievement of the molecular response and its subsequent loss. For patients who had not yet achieved DMR but had achieved MMR, 12.6% had at least one fluctuation. These patients had a significantly poorer failure-free survival compared to patients with no fluctuation: 82.4% versus 93.2%, respectively. Of patients with DMR and fluctuations, none acquired resistance or *BCR-ABL1* kinase domain mutations. This study demonstrated that unstable MMR was associated with an increased risk of imatinib resistance, whereas fluctuations of deeper molecular responses did not influence outcome.⁶³

Data suggest that when a DMR is achieved it is relatively stable and the risk of TKI resistance is low. However, vigilance and long-term molecular monitoring are recommended, even for patients with stable DMR. A rare case of late relapse associated with the acquisition of a *BCR-ABL1* kinase domain mutation after long-term, stable, undetectable *BCR-ABL1* (MR4.5) has been reported.⁶⁴ A Y253H mutation was first detectable by Sanger sequencing more than 2 years after *BCR-ABL1* transcripts became detectable, which was almost 9 years after commencing imatinib therapy.

Does real-time quantitative polymerase chain reaction analysis provide sufficient sensitivity?

Multiple clinical trials have consistently confirmed that approximately half of the patients who stop TKI in a stable DMR have molecular recurrence.⁷⁻¹⁶ Despite many years having passed since results of the first discontinuation trials were reported, reliable prediction of molecular relapse has eluded researchers. The NCCN provides criteria for attempting TKI discontinuation, which include stable MR4 (*BCR-ABL1* ≤0.01%) for at least 2 years.²⁰ The French CML Study Group recommends MR4.5 (*BCR-ABL1* ≤0.0032%) for at least 2 years.¹⁷ Although the difference in *BCR-ABL1* levels seems minor, the slow kinetics of the *BCR-ABL1* decline means that MR4.5 may not be reached until many months, or even years, after MR4.^{22,65} In the German CML-Study IV the estimated median time to reach MR4 was 3.1 years, and that to reach MR4.5 was 4.9 years.²² Horn *et al.* assessed the transcript dynamics of patients treated with 400 mg imatinib.⁶⁵ They estimated that the median time to reach MR4 was 5.3-6.5 years while that to reach MR4.5 was 9.1-10.7 years. Similarly,

Table 1. Molecular response milestones according to international recommendations and guidelines.

Milestones	ELN 2020 ¹⁹ Optimal	NCCN 2020 ²⁰ TKI-sensitive disease	ELN 2020 ¹⁹ Warning	NCCN 2020 ²⁰ Possible TKI resistance	ELN 2020 ¹⁹ Failure	NCCN 2020 ²⁰ TKI-resistant disease
3 months	≤10%	≤10%	>10%	>10%	>10% if confirmed within 1-3 months	NA
6 months	≤1%	≤10%	>1-10%	NA	>10%	>10%
12 months	≤0.1%	≤1%	>0.1-1%	>1-10%	>1%	>10%
≥15 months		≤1%		NA		>1%
Any time	≤0.1% or ≤0.01% with the aim to achieve TFR		>0.1-1%, loss of MMR indicates failure after TFR		>1%, resistance mutations and high-risk ACA	

ELN: European LeukemiaNet; NCCN: National Comprehensive Cancer Network; TKI: tyrosine kinase inhibitor; NA: not applicable; TFR: treatment-free remission; MMR: major molecular response; ACA, additional chromosome abnormalities in Philadelphia-positive cells.

for 528 patients treated with imatinib doses of 400, 600 or 800 mg in the first-line setting, we found an approximate 3-year difference between the times to reach MR4 and MR4.5⁶⁶ (Figure 1). These studies indicate that the criteria used for TKI discontinuation significantly influence the timing of discontinuation.

A recent meta-analysis of 12 TKI discontinuation trials assessed the factors that influenced the rate of TFR.⁶⁷ Trials with a molecular criterion of MR4.5 or better for selecting patients for TKI discontinuation documented higher rates of TFR at 24 months than those with a criterion of MR4.0: 57.2% versus 50.5%, respectively. This finding could be associated with the longer treatment duration needed to achieve MR4.5 or better. Longer treatment duration is associated with a higher probability of sustaining TFR.¹⁵ It could also indicate that lower

BCR-ABL1 values before TKI cessation increase the chance of maintaining TFR. However, Ross *et al.* failed to predict TFR using a highly sensitive patient-specific DNA-based quantitative PCR technique (sensitivity 10^{-6.2}).⁶⁸ None of the patients studied had detectable *BCR-ABL1* mRNA transcripts, with a sensitivity of MR4.5, at TKI discontinuation. The DNA technique detected *BCR-ABL1* in almost all patients, irrespective of whether TFR was maintained or not. Furthermore, DNA *BCR-ABL1* continued to be detectable in remission. In a follow-up of nine patients in long-term TFR, residual DNA *BCR-ABL1* was detected in most patients. In two of these nine patients, DNA *BCR-ABL1* was persistently detectable at every measurement time-point over 6.5 and 10.5 years, and the level declined over time.⁶⁹ The authors hypothesized that this could indicate gradual extinction of long-lived lineage-committed cells that lack self-renewal capacity or depletion of slowly proliferating leukemic precursor cells. In a recent analysis that included the same patients, the lineage of residual leukemic cells in patients who sustained TFR was investigated.⁷⁰ Residual DNA *BCR-ABL1* was detected predominantly in the lymphoid compartment and never in granulocytes. This was an important finding since the study demonstrated that the detection of residual *BCR-ABL1* may not imply the persistence of multipotent leukemic cells. Additionally, lymphocytes were found to be part of the leukemic clone at the time of diagnosis of CML and *BCR-ABL1* was expressed in both RNA and DNA. This may have implications for studies in which T cells are used as a source of non-leukemic cells to establish the somatic status of variants in next-generation sequencing studies.⁷¹

Digital PCR is being increasingly used to measure residual *BCR-ABL1* DNA and RNA and may improve the precision and sensitivity of detection.^{13,72-82} Goh *et al.* improved the sensitivity of *BCR-ABL1* transcript detection by pre-amplification prior to digital PCR,⁷² thereby introducing a semi-quantitative nested PCR approach.⁸³ Importantly, *BCR-ABL1* transcripts were detected by digital PCR in patients with stable undetectable *BCR-ABL1* using the standard qRT-PCR method. This observation

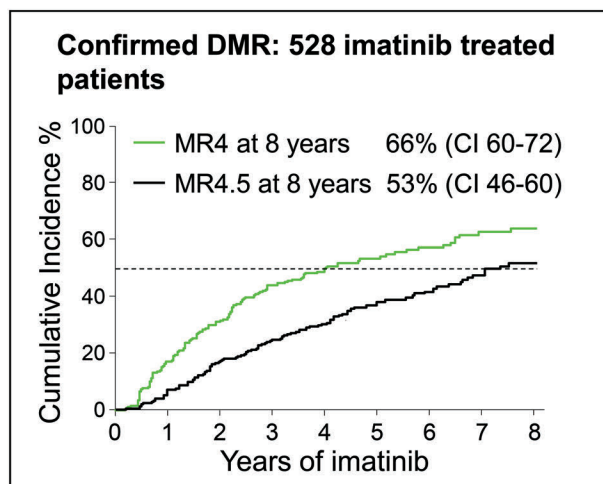


Figure 1. Time difference between achieving MR4 and MR4.5. We determined the cumulative incidence of achieving confirmed MR4 and MR4.5 in 528 patients consecutively treated in clinical trials of imatinib. There was an approximate 3-year difference in the median time to reach the deep molecular response levels in the total cohort. DMR: deep molecular response; CI: confidence interval.

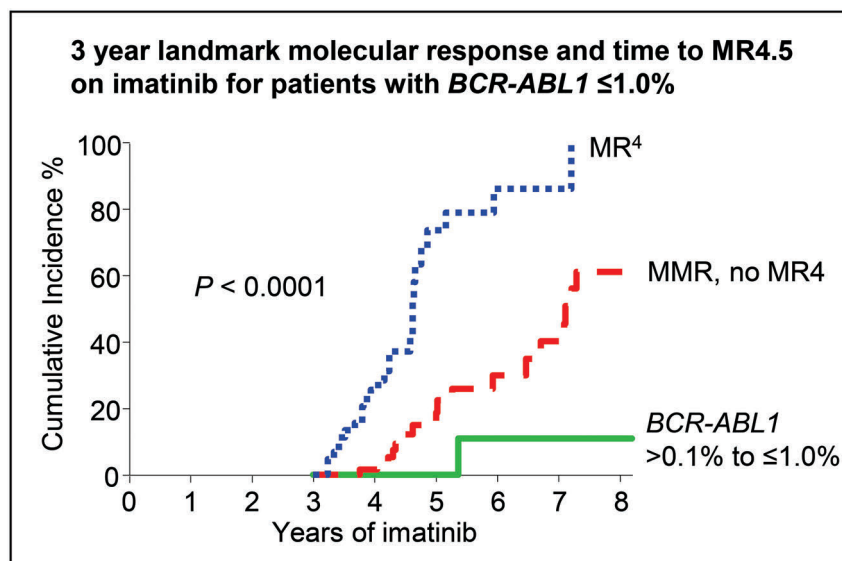


Figure 2. Patients with MR4 at 3 years of imatinib treatment have a high probability of reaching MR4.5 with continued imatinib. Patients without a major molecular response or MR4 at 3 years of first-line imatinib therapy may benefit from a switch to a more potent tyrosine kinase inhibitor if the goal of therapy is to achieve MR4.5.⁶⁶ MMR: major molecular response.

corroborates the studies reporting residual DNA *BCR-ABL1* in similar patients' samples.^{68,84}

A critical factor for the reliability of data when using very sensitive PCR is controlling for contamination and false positive results. This is overcome by quantification of genomic DNA *BCR-ABL1* breakpoints that are unique to each patient. However, RNA-based techniques are prone to contamination since common sequences are amplified for each patient. Negative controls for each step of the assay are essential to detect contamination and to establish the threshold for positivity. Optimization of a digital droplet PCR (ddPCR) method ensured a background false-positive rate of only 5% of samples and reliably detected *BCR-ABL1* transcripts to MR5 (0.001%).⁷⁸ Another group carefully evaluated the positivity threshold of a ddPCR method by testing 30 samples from non-CML patients.⁷⁹ The method was used to measure residual disease in a substantial proportion of patients enrolled in the Stop Imatinib 2 (STIM2) study prior to TKI discontinuation. The patients all had undetectable *BCR-ABL1* transcripts by qRT-PCR at a sensitivity level of MR4.5 for at least 2 years. Using the more sensitive ddPCR method, a cut-off value for detectable residual disease was established to predict TFR. A conversion factor to the IS was also established. Patients with residual *BCR-ABL1* levels above the threshold of 0.0023% IS had a higher probability of molecular recurrence at both 6 and 12 months after TKI discontinuation: 66% versus 44% and 68% versus 46%, respectively. However, purely on the basis of ddPCR positivity versus negativity, there was no predictive power for disease recurrence.⁷⁹

Whether or not digital PCR can reliably identify the patients destined for TFR requires further analysis and at this stage the technique should not be used to select patients for a TFR attempt.^{79,85} Key factors for resolution are harmonization of methods and standardization to the IS. Importantly, laboratories must ensure that threshold levels above the background noise are carefully implemented. Furthermore, based on the ddPCR data from the STIM2 trial,⁷⁹ Yan *et al.* estimated that if the ddPCR cut-off of <0.0023% IS had been added to the STIM2 entry criteria, the rate of TFR at 12 months would have been 54% instead of the reported 49% for the patients tested by ddPCR.⁸⁵ Furthermore, some patients capable of maintaining TFR would be excluded. The authors of the STIM2 ddPCR study clearly stated that this degree of improvement was insufficient for implementation of ddPCR for selection of patients.⁷⁹ However, digital PCR may become an important complement to qRT-PCR in decisions for attempting TFR, in particular, using a ddPCR method that reports *BCR-ABL1* values on the IS.⁸⁶

Factors other than the depth of *BCR-ABL1* response at the time of stopping TKI may influence sustained TFR. The duration of DMR before stopping treatment and longer duration of therapy were factors in the EURO-SKI study, which is the largest discontinuation trial.¹⁵ The detection of *BCR-ABL1* transcripts using sensitive PCR in patients with TFR demonstrates that elimination of the leukemic clone may not be necessary for sustained TFR.⁷² Immune surveillance may be an important factor.^{87,88} Sustained MR4.5 is a reasonable molecular response for a TFR attempt and methods should aim to reliably detect MR4.5, irrespective of whether the method used is qRT-PCR or digital PCR.

Strategies to improve the rates of MR4.5

The first-line use of the second-generation TKI nilotinib and dasatinib is associated with higher rates of MMR and MR4.5 than the rates following the use of imatinib and the responses are achieved earlier.^{89,90} However, deeper molecular responses did not translate into improved survival. Strategies have been explored to induce deeper molecular responses for imatinib-treated patients, including switching to a more potent inhibitor. The ENESTcmr clinical trial was a randomized study for imatinib-treated patients in complete cytogenetic response with detectable *BCR-ABL1*.^{91,92} Patients continued on imatinib or switched to nilotinib 400 mg twice daily. The primary endpoint of the study was undetectable *BCR-ABL1* MR4.5 at 12 months. Higher rates of MMR and MR4.5 were achieved with nilotinib, although adverse events were more common. The cumulative incidence of MR4.5 following the switch to nilotinib was 32.7% at 12 months and 42.9% at 24 months.⁹¹ The cumulative incidence of MR4.5 among patients who continued imatinib therapy was 13.5% and 20.8% at 12 and 24 months, respectively. Consistent with other trials, cardiovascular events were more frequent among the nilotinib-treated patients.⁹² The potential for improved molecular responses with more potent TKI must be assessed in light of the potential for cardiovascular events.⁹³ Furthermore, with the high cost of second-generation TKI in many countries, the incremental benefit of using these inhibitors to achieve DMR may not provide good value.⁹⁴ The ELN had considerable discussion when revising the recommendations for managing CML in regards to the advisability of using a second-generation TKI in the first- or second-line setting to achieve DMR. However, there was no final consensus.¹⁹

We determined whether there was a level of *BCR-ABL1* in imatinib-treated patients below which a switch to a more potent inhibitor may not be necessary in order to reach a timely MR4.5.⁶⁶ Among 528 patients consecutively treated in clinical trials of imatinib, 147 had achieved a complete cytogenetic response, or its molecular equivalent of $\leq 1.0\%$ *BCR-ABL1*,⁹⁵ at 3 years of imatinib treatment. None of these patients had achieved MR4.5 at that time. Landmark analyses demonstrated that the patients without MMR at 3 years of imatinib therapy had a negligible probability of achieving MR4 or MR4.5 with up to 5 additional years of imatinib (Figure 2). These patients may benefit from a switch to a more potent inhibitor in order to achieve DMR. Similarly, patients with MMR but not MR4 at 3 years of imatinib therapy had a significantly lower cumulative incidence of MR4.5 compared to patients with MR4: MMR versus MR4, 61% versus 100% by 5 years after the landmark ($P < 0.0001$). However, most patients with MR4 at 3 years of imatinib therapy did indeed achieve MR4.5 with 2 additional years of imatinib treatment. These findings may help clinical decisions when considering a switch of treatment to optimize TKI discontinuation options, while minimizing the additional risk of adverse events with more potent TKI.

Conclusion

Reaching a DMR is now considered a goal of therapy by many clinicians. The importance of a DMR for patients aiming to achieve TFR is recognized by the inclusion of DMR as an endpoint measure in clinical trials.

Additionally, TFR is increasingly acknowledged as an avenue to save money in over-stretched healthcare budgets.^{15,96} However, not all patients and clinicians consider TFR as the goal of therapy.⁹⁷ Prolonged survival with minimal side-effects are equally important goals and the patient's choice is central for treatment decisions.

The take-home messages from this review are: (i) it can take many years to reach a DMR for some patients and earlier achievement is possible with a second-generation inhibitor; however, there is no consensus on the benefit of using a second-generation inhibitor to achieve a DMR and therapy choices must be made in the context of the

individual patient's comorbidities; (ii) a sustained MR4.5 prior to treatment discontinuation may be associated with higher rates of TFR than sustained MR4; and (iii) deeper molecular responses have so far not reliably predicted TFR. Regardless, sustained DMR is certainly a desirable outcome since it is associated with an extremely low risk of loss of response and TKI resistance, and is mandatory for any patient considering TFR.

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